

AMENDMENTS TO THE SPECIFICATION

On page one of the specification, before the heading "Technical field of the invention", please add the following new paragraph:

-- This application is the National Stage of PCT/DE99/02601 filed on August 16, 1999. --

Please replace the paragraph beginning at page 2, line 26 and extending through page 3, line 9 with the following amended paragraph:

-- Furthermore, no antibodies to the human sGC α 1/ β 1 are currently available that are monospecific, directed against the human sequences, or that have been shown to be suitable for immunoblots with human tissues. Peptide antibodies reported thus far only partially show these features: Harteneck et al. and Guthmann et al. used a peptide sequence (VYKVETVGDKYMTVSGLP; SEQ ID NO: 11) that is highly conserved in guanylyl cyclases. Therefore, cross-reaction with particulate guanylyl cyclases (e.g. GC-C) can be expected. Guthmann et al. used a peptide sequence (YGPEVWEDIKKEA; SEQ ID NO: 12) identical to hsGC β 1 and a peptide sequence (KKDVEEANANFLGKASGID; SEQ ID NO: 13) identical to hsGC α 1 except for two amino acid exchanges. However, the function of these antibodies in immunoblots was only shown for *enriched* hsGC from human platelets. In addition, the antisera to hsGC α 1 detect a second, unspecific product. Humbert et al. and Koesling et al. used a peptide sequence (SRKNTGTEETE QDEN; SEQ ID NO: 14) of bovine sGC β 1 that partially (amino acids 1-10) overlaps with the peptide used here for the human sGC β 1 (amino acids 13-22) and is identical in this region. The C-terminus of this bovine peptide (amino acids 11-15), however, is clearly different from the human sequence. On the other hand, the antiserum to this peptide was not tested with a human protein but rather only used for immunoprecipitation of the bovine sGC. --

Please replace the paragraph beginning on page 7, line 30 and extending through page 8, line 5 with the following amended paragraph (PLEASE NOTE: Under lining of "Figures 5 and 6" on page 7, line 30 was underlined in the original text and is not meant to be interpreted as added text in the amended paragraph below):

-- Figures 5 and 6 show the baculovirus transfer vectors pVL1393 and pAcG2T, respectively (both without the hsGC cDNA), which were used for the construction of recombinant baculoviruses for the expression of human sGC α 1/ β 1 in Sf9 cells. The circular plasmid with the restriction sites (short names and position in base pairs), the gene for ampicillin resistance (Amp^R), the 'origin of replication' (ColE ori), the polyhedrin promotor, the glutathione-S-transfease sequence (only in Fig. 6), and the multiple cloning site (MCS) are shown at the top of the figures. Figure 5 shows the multiple cloning site with its unique restriction sites at the bottom (upper DNA strand = SEQ ID NO: 15; lower DNA strand = SEQ ID NO: 16). Figure 6 shows the multiple cloning site with the unique restriction sites as well as a thrombin cleavage site ~~normal~~ at the bottom (DNA sequence = SEQ ID NO: 17; amino acid sequence = SEQ ID NO: 18, thrombin cleavage site amino acid sequence = SEQ ID NO: 19). --

Please replace the paragraph on page 8, lines 7-18 with the following amended paragraph (PLEASE NOTE: Under lining of "Figure7" on page 8, line 7 was underlined in the original text and is not meant to be interpreted as added text in the amended paragraph below):

-- Figure 7 shows the construction of the plasmids hsGC β 1-pVL1393 (without GST-tag) with the hsGC β 1 cDNA, which was used to obtain genetically modified, hsGC β 1-expressing baculovirus by homologous recombination. The procedure for the plasmid pAcG2T-hsGC β 1 (with GST-tag = glutathione-S-transferase cDNA from *Schistosoma japonicum*) is identical.

A fragment was produced that carries an additional BamHI-site at its 5' end by means of PCR with the primers A (bases 89-116 of the hsGC β 1 cDNA + BamHI site at its 5' end; SEQ ID NO: 9) and B (bases 692-711 of SEQ ID NO: 3, the hsGC β 1 cDNA [noncoding strand] with natural KpnI site). Due to the additional restriction sites, fragment 1 (PCR fragment with new BamHI site) and fragment 2 (hsGC β 1 cDNA from KpnI site to EcoRI site) could be inserted together into the BamHI and EcoRI sites of the plasmid pVL1393. Thus, the complete hsGC β 1 cDNA is under the control of the polyhedrin promotor (PHP). --

Please replace the paragraph on page 8, lines 20-31 with the following amended paragraph (PLEASE NOTE: Under lining of "Figure 8" on page 8, line 20 was underlined in the original text and is not meant to be interpreted as added text in the amended paragraph below):

-- Figure 8 shows the construction of the plasmid hsGC α 1-pVL1393 (without GST-tag) with the hsGC α 1 cDNA which was used to obtain genetically modified, hsGC α 1-expressing baculovirus by homologous recombination. The procedure for the plasmid pAcG2T-hsGC α 1 (with GST-tag = glutathione-S-transferase cDNA from *Schistosoma japonicum*) is identical. A fragment was produced that carries an additional BamHI site at its 5' end and a natural BsaAI site within the sequence by means of PCR with the primers C (bases 524-541 of the hsGC α 1 cDNA + BamHI site at its 5' end; SEQ ID NO: 7) and D (bases 1232-1249 of SEQ ID NO: 1, the hsGC α 1 cDNA- [noncoding strand]). Due to the added restriction site, fragment 3 cut with BsaAI (PCR fragment with new BamHI site to the BsaAI site) and fragment 4 (hsGC α 1 cDNA from BsaAI site to EcoRI site) could be inserted together into the BamHI and EcoRI sites of the plasmid pVL1393. Thus, the complete hsGC α 1 cDNA is under the control of the polyhedrin promotor (PHP). --

Please replace the paragraph beginning on page 15 at line 18 and continuing through page 16, line 6 with the following amended paragraph:

-- By use of the peptide antibodies to sGC of the present invention, it is possible to determine the expression of sGC in human tissues as well as diagnose dysfunctional conditions (if expression of sGC is too high, too low, or absent). In addition, the present invention provides the technical prerequisites needed to further elucidate the control of transcription and translation of hsGC. The peptide antibodies of the invention have the advantage that they are monospecific, directed at the human sequence, and that their suitability for immunoblots with human tissues has been demonstrated. Other peptide antibodies exhibit these features only partially: Harteneck et al. and Guthmann et al. used a peptide sequence (VYKVETVGDKYMTVSGLP; SEQ ID NO: 11) that is relatively highly conserved in guanylyl cyclases. Thus, cross-reaction with particulate guanylyl cyclases (e.g. GC-C) would be expected. Furthermore, Guthmann et al. used a peptide sequence (YGPEVWEDIKKEA; SEQ ID NO: 12) identical to hsGC β 1 and a peptide sequence identical to hsGC α 1 except for two amino acid exchanges (KKDVEEANANFLGKASGID; SEQ ID NO: 13), but the function of these antibodies in immunoblotting has only been shown for enriched hsGC from human platelets. In addition, these antibodies to hsGC α 1 recognized a second, unspecific product. Humbert et al. and Koesling et al. used a peptide sequence (SRKNTGTEETEQDEN; SEQ ID NO: 14) from bovine sGC β 1 that is in part (amino acids 1-10) identical to the peptide used here (amino acids 13-22) for hsGC β 1, although the C-terminus (amino acids 11-15) differed markedly from that of the human sequence. The antiserum to this peptide, however, has not been tested on human protein and has only been used for immunoprecipitation of bovine sGC. --

Please replace the paragraph on page 20, lines 5-23 with the following amended paragraph:

-- The expression of sGC α 1 and sGC β 1 mRNA in human tissues was demonstrated by means of PCR (Figure 4). The amplification of a hsGC β 1 fragment with a PCR primer pair (5'-AAAAGGATCCATGTACGGATTTGTGAAT-3' = nucleotides 89-106 of SEQ ID NO: 3, the hsGC β 1 cDNA sequence with added restriction site; 5'-ATGCGTGATTCCTGGGTACC-3' = 692-711 of SEQ ID NO: 3, the hsGC β 1 cDNA sequence) with an annealing temperature of 54°C resulted in one specific band each in cDNA libraries from brain, heart, kidney, lung, pancreas, and skeletal muscle. The identity of the amplified fragment was confirmed by sequencing. The amplification of a hsGC α 1 fragment with a PCR primer pair (5'-AAAAGGATCCATGTTCTGCACGAAGCTC-3' = nucleotide 524-541 of SEQ ID NO: 1, the hsGC α 1 cDNA sequence with added restriction site; 5'-ATTATGGAAGCAGGGAGG-3' = 1249-1232 of SEQ ID NO: 1, the hsGC α 1 cDNA sequence) with an annealing temperature of 54°C resulted in one specific band each in cDNA libraries from heart (Figure 4A) and lung (not shown). In each case, the sequencing of the fragments resulted in the corrected hsGC α 1 sequence; and the 'hsGC α 3' sequence published by Giuili et al. was not found. Thus, it was shown that in humans, only one hsGC α 1/ β 1 exists, and that the potential hsGC α 3/ β 3 is a result of sequencing errors. This results in a clear picture for cardiovascular research concerning which sGC isoform should be the target protein for pharmacological screening. --